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ABSTRACTS OF PAPERS

Twenty-first Annual Albert L. Tester Memorial Symposium, 11–12 April 1996[1]

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester, who, at the time of his death in 1974, was senior professor of zoology at the University of Hawai'i at Mānoa. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields in biology. Papers reporting original research in all aspects of biology, solicited from graduate students at the University, are presented at the spring-semester symposium. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawai'i Foundation provides two prizes for the best papers. Judges include representatives of the Department of Zoology faculty, winners from the preceding symposium, and a distinguished scholar from another university, who also presents a major symposium address. In 1996 George L. Gabor Miklos of the Neurosciences Institute, La Jolla, California, participated in the symposium. In 1997 Stephen A. Wainwright, Professor of Zoology, Duke University, Durham, North Carolina, participated in the symposium.

Efficiency Analysis of an Asian Shrimp Industry: Malaysian Shrimp Culture[2]

L. H. P. GUNARATNE[3]

Though shrimp production in Asia has been tremendously increased during the past decade because of intensive technology, the sustainability of the industry is under severe threat as a result of environmental deterioration. However, some of the shrimp-producing countries in the region, such as Malaysia, still show a trend toward intensification. With this background, efficiency of resource use in the Malaysian shrimp industry was examined. The production frontiers and productive efficiencies of 77 intensive and semi-intensive shrimp farms in Malaysia were estimated using stochastic frontier techniques. The industry exhibited constant returns to scale with a high output elasticity of 0.859 for feed. The analysis further revealed that intensive farms were not significantly more efficient compared with semi-intensive farms. In both systems, the recommended optimum input level for each input was lower than the average actual use, except for labor. The results can be used to develop policy guidelines to address the sustainability of the shrimp industry in the Asia-Pacific region.

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[2] I acknowledge Network for Aquaculture Centres in Asia-Pacific for data.

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Hawaiian *Tetragnatha* Spiders: High Species Diversity from Two Colonizations and Within-Archipelago Diversification

GERALD L. HASTY, ROSEMARY G. GILLESPIE, AND GEORGE K. RODERICK[4]

The Hawaiian Archipelago contains several of the world's most renowned examples of terrestrial species radiations. Many have been inferred to be the result of single colonizations, with subsequent geographical and ecological isolation important to speciations. We have investigated endemic Hawaiian spiders of the orb-weaving genus *Tetragnatha*, family Tetragnathidae, as one set of organisms specially suited for studies of Hawaiian evolutionary ecology. The ca. 300 species of *Tetragnatha* outside Hawai'i compose a morphologically, ecologically, and behaviorally conservative group. Within Hawai'i, however, the 50–60 species in collections exhibit a range of morphologies, habitat associations, and foraging behaviors. Many have abandoned web-building and are roaming or ambush predators. We sequenced 325 bp of the cytochrome oxidase subunit I gene from the mitochondrial DNA of 14 endemic Hawaiian *Tetragnatha* species (from Kaua'i, O'ahu, Maui, and Hawai'i), two non-Hawaiian *Tetragnatha* species (from China and Australia), and one species of *Araneus* (family Araneidae, introduced from North America). A cladistic parsimony analysis supported three major conclusions: (1) The Hawaiian *Tetragnatha* are paraphyletic, the result of colonizations by two different lineages; (2) The abandonment of web-building in extant species has a single evolutionary origin; and (3) The persistence of closely related taxa within this radiation is dependent upon interisland dispersal. These results add to our growing knowledge of this recently discovered island radiation. It is likely that the relatively enhanced geographical and ecological separation of *Tetragnatha* populations in Hawai'i compared with those on continents has contributed to the remarkably high species diversity found within this genus across the Islands.

Molecular Characterization of an *engrailed*-like Homeobox from the Sepiolid Squid *Euprymna scolopes*[5]

PATRICIA N. K. L. LEE[6]

The *Drosophila* segment-polarity gene, *engrailed (en)*, contains a 180-base pair region of highly conserved sequence homology known as the homeobox. This encodes a helix-turn-helix motif involved in DNA binding and transcriptional regulation. Within the arthropods, annelids, and vertebrates, *en* is involved in pattern formation, specifically in demarcating compartment boundaries. *En* expression studies also point to a conserved role during the differentiation of neurons in the central nervous system. At the start of

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[5]Supported by Sigma Xi Grants-in-Aid of Research, the Hawaiian Malacological Society, and a Seed Money Grant by the University Research Council to H. G. de Couet.

[6]Department of Zoology, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822. Sponsor: Dr. H. G. de Couet.

this project, the *en* gene had not been examined in the nonsegmented mollusks. Recently, *en* homologues have been cloned from representatives of all five molluscan classes. Within the cephalopods, two *en* homologues were amplified from *Nautilus pompilius,* but attempts at cloning *en* from a coleoid cephalopod *(Loligo)* were unsuccessful. Using PCR with degenerate primers, we have amplified a 232-base pair *en*-like sequence *(ES-en)* from the sepiolid squid *Euprymna scolopes* (Berry, 1913). Only one *en*-like sequence was characterized from *E. scolopes.* This sequence lacks the intron found in the *Drosophila en* homeobox. The putative amino acid sequence contains all conserved amino acids found specifically in members of the *en*-class of homeobox genes. *ES-en* shares 58% nucleotide identity with the *Drosophila en* homeobox. Among mollusks, *ES-en* has highest homology to *Ilyanassa obsoleta* and *N. pompilius* A *en*-like sequences (67% and 65%, respectively). At the amino acid level, *ES-en* is 75% identical to the *Drosophila en* homeodomain. *ES-en* shares the highest identity (81%) with *N. pompilius* A, but only 61% with *Cadulus fusiformis.* The presence of this "segmentation gene" in unsegmented organisms is interesting, but not surprising. Although the role of *en* in segmentation was the first to be recognized, it appears that the ancestral function was in the differentiation of specific cell types in the central nervous system. It has been suggested that this function was expanded within the arthropods, annelids, and vertebrates to include the control of compartment-specific differentiation, as seen in segmentation.

An Inventory of Native Brook Trout (*Salvelinus fontinalis*) Populations along the Upper West Branch of the Susquehanna River

ROBERT LOVICH[7]

Native brook trout are widely distributed in suitable habitat in Pennsylvania. Over 200 km of streams in the upper West Branch of the Susquehanna River were surveyed periodically from 1981 to 1995 for the presence of brook trout. Brook trout were found in 68% of the stream distance surveyed, including 66% of the first-order stream segments. Higher-order streams were more likely to be occupied by brook trout and had proportionately more stream distance of brook trout habitat than lower-order streams. Few other fish species were collected during the survey. The primary cause of brook trout extirpation in the area is acid mine drainage from the surrounding bituminous coal mines that occupy substantial portions of the regional landscape. Atmospheric deposition of strong acids in these poorly buffered streams has undoubtedly contributed to the extirpation of brook trout populations. In a subset of steams that did not contain brook trout, pH ranged from 3.8 to 4.7. Unpolluted headwater streams provide important refugia for recolonizing the polluted but recovering West Branch of the Susquehanna River and its major tributaries.

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Responses of Mesozooplankton Abundance, Grazing, and Community Structure to a Mesoscale Iron Enrichment Experiment in the Eastern Equatorial Pacific

GRETCHEN C. ROLLWAGEN[8]

On 29 May 1995 (YD 149) a 72-km^2 area of surface ocean located at 3° S, 104° W was enriched with acidic iron sulfate plus the inert tracer SF_6 and subsequently tracked and monitored for 17 days. The biological response to iron addition was significant, with a nearly 30-fold increase in surface chlorophyll *a* concentration over preinfusion levels on YD 156, accompanied by a 40% decrease in surface nitrate concentration. Both parameters returned to near normal (preinfusion) levels by YD 165. Mesozooplankton (0.2–2.0 mm) biomass, abundance, grazing impact, and vertical community structure were measured, both in and out of the patch, before iron infusion and periodically throughout the enrichment experiment. Midday and midnight net tows were taken obliquely through the surface mixed layer, and vertical net tows were taken to five overlapping depths down to 200 m to determine the depth distribution of populations. Mean mesozooplankton carbon biomass from "control" sites remained fairly constant (day = 3.3 mg C m^{-3}, night = 4.1 mg C m^{-3}) during the 17-day experiment. However, mean daytime biomass increased significantly inside the patch to 8.6 mg C m^{-3}, whereas mean nighttime biomass showed little change (mean = 4.6 mg C m^{-3}). Small (<0.5 mm) calanoid copepods, primarily of the families Paracalanidae and Pseudocalanidae, accounted for the majority of the daytime biomass increase, with a nearly 4-fold increase in the mixed layer over control levels on YD 157. Abundance data from the depth-stratified tows suggest that the daytime increase in calanoid copepods resulted from upward vertical migrations from the lower euphotic zone to the mixed layer. Mesozooplankton grazing upon phytoplankton, measured as gut fluorescence, also increased significantly inside the patch relative to outside controls both during the day (23-fold increase) and night (24-fold increase). However, because of the concurrent increase in mixed-layer chlorophyll *a* concentration, mesozooplankton grazing impact (percentage of chlorophyll consumed day 1) only increased by 53% inside the patch relative to control levels. These results show that mesozooplankton communities respond to iron-induced phytoplankton blooms through shifts in vertical community structure and increases in grazing rates. However, because the grazing increases are more or less in proportion to the increase in phytoplankton standing stock, mesozooplankton play a limited role in suppressing the response of phytoplankton to iron enrichment.

Distribution, Reproduction, and Diet of the Gray Reef Shark, *Carcharhinus amblyrhynchos,* in Hawai'i

BRADLEY M. WETHERBEE[9]

Distribution, reproduction, and diet of the gray reef shark, *Carcharhinus amblyrhynchos* (Bleeker, 1856), were examined using data collected during shark control programs in the main Hawaiian Islands (MHI) and during research fishing in the

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Northwestern Hawaiian Islands (NWHI). A total of 469 sharks was caught between 1967 and 1980. Gray reef sharks had a restricted distribution in the MHI, occurring only in the vicinity of Ni'ihau and Molokini Islands, but were one of the most abundant sharks throughout the NWHI. Catch rate was higher for males than for females in standard longline fishing at all locations and during all seasons. Depth distribution of males and females was similar, although females were more common at shallower depths. Males ranged in size from 79 to 185 cm total length (TL) and matured at between 120 and 140 cm TL. Females ranged in size between 63 and 183 cm TL and matured at about 120 cm TL. Litter size ranged from three to six with an average of 4.1 pups. Size at birth was estimated to be just over 60 cm TL. Most gray reef sharks (85%) consumed teleosts, but some sharks fed on cephalopods (29.5%), and crustaceans (4.9%). Larger sharks fed less frequently on teleosts and more frequently on cephalopods.

Twenty-second Annual Albert L. Tester Memorial Symposium,
6–7 April 1997[10]

Kinetic Variability in Inactivation-Removed *Shaker* B K^+ Channels

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We have assessed the kinetic stability of inactivation-removed *Shaker* K^+ channels expressed in *Xenopus* oocytes by patch clamp technique in cell-attached, inside-out, and "crammed-in" patches. We observed the following changes over time: (1) a decrease in the rate of activation and (2) a decrease in the rate of deactivation. Whereas most on-cell patches are stable, inside-out patches show significant parameter changes within the first 10 min at room temperature. The changes outlined above occur independently of each other and are partially reversible by cramming the patch back into the intracellular milieu of the oocyte. Using on-cell recordings, we found that there are variations in both kinetics and voltage sensitivity between different patches from the same oocyte. Our observations imply that both the voltage sensitivity and rates of activation and deactivation may be separately and reversibly regulated by metabolic processes within the nonhomogeneous intracellular environment of the oocyte.

[10] Manuscripts accepted 10 April 1997.

[11] Biomedical Sciences Interdisciplinary Program (Dr. Martin D. Rayner), Bekesy Laboratory of Neurobiology, Pacific Biomedical Research Center, University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822.

Kinorhyncha: First Record of This Interstitial Phylum for Hawai'i[12]

STEPHANIE R. BAILENSON[13]

Very little has been added to our knowledge of members of the phylum Kinorhyncha (Reinhard, 1881) since the first were described by Dujardin from off the Normandy coast in 1841. Less than half a millimeter in length, kinorhynchs are often overlooked during sediment samplings. This was probably the case in Hawai'i until recently. In 1984, nine specimens were collected in a single sand sample from a 70-m-deep sewage outfall. Others were not seen again until 1996. Specimens have been collected from that outfall plus two others (at 30 m and 70 m depth) off O'ahu. Using light and scanning electron microscopy, taxonomy down to the generic level has been determined. Their 16 placids and lateral and middorsal spines place these kinorhynchs in the order Cyclorhagida Zelinka, 1896. The complete ring of cuticle for the third zonite places them in the suborder Cyclorhagae Zelinka, 1896. Absence of a midterminal spine in the adults places them within the family Echinoderidae Bütschli, 1876, and the genus *Echinoderes* Claparéde, 1863. Taxonomy beyond the genus level is in need of serious revision and has, thus far, prevented species designation of these specimens.

A Polychaete Detective Story: Which Species of *Hesionura* (Phyllodocidae) Is Found in Hawai'i?[14]

BRENDAN M. BARRETT[15]

Benthic community studies at four O'ahu sewage outfalls (Barbers Point, Sand Island, Wai'anae, and Mōkapu) have focused on sediment-dwelling infauna. Sediment samples are collected, preserved, and then elutriated to separate the organisms from the sediment, and the invertebrates are sorted, identified, and counted. These data are used for statistical analyses that yield insights into the dynamics of the benthic communities at those sites. Polychaetes, the most abundant and speciose soft-bodied invertebrate taxa at those sites, are identified to the lowest taxon possible, but many species are undescribed or not reported from Hawai'i. Search of the current literature has provided tentative identification of such a species. In this case, the characteristics that led to the placement of the Hawaiian specimens in the genus *Hesionura* Hartmann-Schröder are an elongated prostomium with four antennae and absence of a nuchal papilla. Furthermore, they have three pairs of tentacular cirri with one pair on segment 1 and the other two pairs on segment 2. The second pair is smaller than the third. The dorsal cirri of segment 3 are absent, but setae are present. The Hawaiian specimens are closest to an Australian species of *Hesionura* because of the type and arrangement of setae. The superiormost seta of each parapodium has a trifid shaft rostrum, whereas the rostra of the remaining three

[12] I acknowledge the Department of Zoology and the Water Resources Research Center for their funding, Dr. Julie Bailey-Brock, and the wormlab.

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[14] I acknowledge the Water Resources Research Center, and Dr. J. H. Bailey-Brock, for support and encouragement of research in the wormlab.

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setae are bifid. The second most superior seta has a long serrate blade, and the other setae have smaller denticulate blades. The evidence gained from the literature coupled with other sources, such as scanning electron microscopy and advice from specialists focusing on the family, should lead to the identification of the species.

Morphology of the Beryciform Pelvic Girdle[16]

ERIN BAUMGARTNER[17]

Interrelationships of the diverse order Beryciformes are controversial. Pelvic girdle characters have been used to hypothesize that the family Holocentridae does not belong within Beryciformes, but is, rather, the sister group of the higher Percomorpha. A total evidence analysis combining the eight pelvic girdle characters examined in this study with data from other recent research on beryciform relationships revealed a sister-group relationship between the families Berycidae and Holocentridae. This total evidence analysis also showed the suborder Stephanoberycoidei as polyphyletic, with the family Melamphaidae not being placed with the family Stephanoberycidae. The traditional suborder Berycoidei was shown as monophyletic, and a relationship between holocentrids and higher percomorphs was not supported. Examination of characters that have supported a holocentrid–higher percomorph relationship indicated that similarities uniting holocentrids with higher percomorphs are probably independently derived.

Diet, Gastric Evacuation, and Daily Ration of Juvenile Scalloped Hammerhead Sharks, *Sphyrna lewini*

AARON BUSH[18]

Gastric evacuation, diet, and daily ration of juvenile scalloped hammerhead sharks, *Sphyrna lewini*, were studied in Kāne'ohe Bay, O'ahu, Hawai'i. Wild sharks were caught in gill nets and stomach contents were analyzed for dietary data, and gastric evacuation data were collected from sharks held in field enclosures. Stomach contents were collected by gastric eversion in all cases. Daily ration estimates were based on stomach contents of wild sharks and digestion rates of captive individuals. Crustaceans made up 73% and 96% of the diet, by percentage number and percentage frequency of occurrence, respectively, and teleost accounted for 16% and 25% of the diet. The most important prey item was the alpheid shrimp *Alpheus mackayi*, which accounted for ca. 35% of the total diet. The pattern of gastric evacuation was curvilinear and was 90% complete in 19.7 hr at 27.1° C and in 28.6 hr at 24.0° C. Estimates of daily ration were low compared with that of other species.

[16] This work was supported by the PEW Foundation, the National Science Foundation, and the American Museum Collection Study Grants Program.

[17] Department of Zoology (David W. Greenfield), University of Hawai'i at Mānoa, Honolulu, Hawai'i 96822.

[18] Department of Zoology (Kim Holland), Hawai'i Institute of Marine Biology, Kāne'ohe, Hawai'i 96744.

Dynamic Population Hybridization and Gene Flow among Promiscuous Species of the Hawaiian Silversword Alliance[19]

VICKIE CARAWAY AND CLIFFORD MORDEN[20]

The evolutionary potential of hybridization has likely been an important factor contributing to the spectacular degree of adaptive radiation in the silversword alliance, a complex of 28 recognized species among three genera endemic to Hawai'i. Creation of new habitats during island formation has led to speciation of unusual varieties of growth forms on all major islands. Although species are cytologically and morphologically well differentiated, naturally occurring intra- and intergeneric hybrids have been found in all zones of sympatry, and at least three species are believed to be of hybrid origin. To assess the level of gene flow in a well-documented hybrid zone, randomly amplified polymorphic DNA (RAPD) markers were used to detect species-specific alleles and allelic variation within species of *Dubautia scabra* (DC) Keck ($n = 14$) and *D. ciliolata* (DC) Keck ($n = 13$). Plants morphologically intermediate to the two species were examined and found to contain F_1, F_2, and later generations, and introgression of alleles is occurring via backcrosses to the parental species.

Metamorphosis and Tube Formation in the Polychaete *Hydroides elegans*[21]

EUGENIO CARPIZO-ITUARTE[22]

Larvae of the polychaete *Hydroides elegans* (Haswell, 1883) were induced to metamorphose with natural biofilms, and the morphogenetic events during metamorphosis were characterized. In addition, larvae were exposed to a wide concentration range of Cs^+ and excess K^+ in seawater, and the metamorphic responses were compared with those to biofilm. Metamorphic response to natural biofilms occurs during the first 15 min, with up to 80% of the larvae secreting primary tubes during the first hour of exposure. Primary tube formation provides attachment of the larvae to a hard substratum. Responses to Cs^+ and excess K^+ were concentration and exposure-duration dependent, and were slow compared with the response to biofilm. Exposure of larvae to 50 mM excess K^+ for 24 hr yielded up to 66% metamorphosis after 48 hr; a 3-hr pulse exposure of larvae to 10 mM Cs^+ caused up to 69% of larvae to metamorphose. Some larvae metamorphosed without forming tubes when exposed to the ions: 35–63% with K^+ and 7.5–13% with Cs^+. The role of K^+ channels in metamorphic signal transduction was explored by application of the K^+-channel blocker TEA alone and in the presence of K^+, Cs^+, or a biofilm. TEA inhibited induction with these three inducers, suggesting the participation of K^+ channels during metamorphic induction. However, the delayed metamorphic response to ions suggests a different mode of action for Cs^+ and K^+ in comparison with the response to biofilm. Separation of morphogenesis from tube formation at settlement provides a useful experimental tool.

[19] Support for this research was provided by a grant from the Evolution, Ecology, and Conservation Biology Program, University of Hawai'i at Mānoa, Honolulu, Hawai'i.

[20] Department of Botany, Evolution, Ecology, and Conservation Biology Program, University of Hawai'i at Mānoa, Honolulu, Hawai'i.

[21] Supported by Office of Naval Research grant no. N00014-95-1015 to Michael G. Hadfield; E.C.-I. is the recipient of a scholarship for graduate studies from Consejo Nacional de Ciencia y Tecnologia/Universidad Autonoma de Baja California.

[22] Kewalo Marine Laboratory. University of Hawai'i, Honolulu, Hawai'i. Sponsor: Michael G. Hadfield.

Recruitment of Freshwater Gobies to Two Streams on the North Shore of Kaua'i

CHARLES CHONG[23]

Hawaiian freshwater gobies, collectively known as 'o'opu, spawn in streams and their larvae are swept out to sea where they develop for several months. The immature fish then reenter freshwater as postlarvae, move upstream, and complete their life cycle in the stream to which they recruited. Because there are few predators of 'o'opu and little evidence of resource limitation in the Hawaiian stream ecosystem, recruitment from the ocean to freshwater may be the most important life cycle event in determining these fishes' population size. The goal of this study was to accurately quantify the number and species of 'o'opu recruiting to a given stream. Modified Breder traps, placed near the mouths of Limahuli and Hanakāpī'ai streams on the north shore of Kaua'i, were set out during daylight hours beginning in April 1994. The traps were checked hourly for newly recruited gobies, which were then counted, identified to species, and released at the end of the sampling period. Sampling was conducted once a week during daylight hours for at least a year at each stream. In addition, 24-hr samples were made seasonally. The catch data indicate that (1) there is a peak in diurnal recruitment in midafternoon at Hanakāpī'ai, (2) seasonal variation exists, with more fish recruiting in the spring to both streams, and (3) Hanakāpī'ai stream received substantially more recruits than Limahuli stream. If continued, regular sampling at various locations around the state using similar protocol will yield additional information needed for resource management and help to determine if these populations are recruitment limited.

Effects of Grazing Fishes on Distribution of Macroalgae in Kāne'ohe Bay, O'ahu[24]

ERIC J. CONKLIN AND SCOTT T. LARNED[25]

Transect surveys on coral patch reefs in Kāne'ohe Bay, O'ahu, showed that populations of most macroalgae are restricted to interiors of reef flats, far from the reef crest. Alternative hypotheses to explain this distribution are that (1) habitat specificity of the algae restrict them to interior regions of reefs, and (2) interior regions of reefs are refugia from grazing by herbivorous fishes; these grazers are presumed to shelter on reef slopes and forage on both reef slopes and outer reef flats. Assays of grazing intensity conducted across reef flats tentatively support the second hypothesis; grazing intensity decreases with increasing distance from reef crests for a relatively palatable alga, *Gracilaria salicornia* Dawson, but does not change for a less palatable species, *Dictyosphaeria cavernosa* (Forskål) Borgesen. *Dictyosphaeria cavernosa* is not restricted to the interiors of reefs and is in fact abundant on reef slopes in many areas, where herbivorous fish biomass is presumably higher than on reef flats. The presence of *D. cavernosa* on both reef flats and slopes may be due to uniformly low grazing intensity on this species in both habitats, and grazing assays corroborate this. In the reef-slope habitat, variation exists within and be-

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[24] Supported in part by the University of Hawai'i Seagrant College Program.

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tween patch reefs in both *D. cavernosa* abundance and grazing intensity. Estimates of herbivorous fish biomass suggest that reef-slope sites with low algal cover and high grazing intensity also have higher herbivorous fish biomass.

Photosynthetic Gas Exchange of New World Fig Fruits: Tradeoffs between Water Loss and Wasp Survival

SUSAN CORDELL[26]

Figs (*Ficus* spp.) and fig pollination wasps (Agaonidae) are highly coevolved mutualists that depend completely on each other for continued reproduction. Many fruits, including fig fruits, have stomates that are active in CO_2 accumulation and regulation of water loss. Recent experimental studies on the energy balance of fig fruits clearly demonstrate that fig pollination wasps are very sensitive to heat and die at temperatures only a few degrees above ambient, indicating the importance of cooling by transpiration. This study was undertaken to evaluate if fig leaves and fruits are under the control of a midday depression, and if so, how transpiration in fig fruits is maintained for wasp survival. To prevent water loss, most photosynthetically active organs undergo a midday depression in which both assimilation and stomatal conductance are drastically reduced. Diurnal gas exchange measurements of two species of fig leaves and fruits revealed a differential relationship between assimilation and stomatal conductance. Leaf photosynthesis exhibited a midday depression between 1200 and 1600 hours, whereas this observance was not a consistent pattern with fruits. The decrease in leaf photosynthesis was in all cases associated with a concomitant decrease in stomatal conductance. Contrary to this result, stomatal conductance of fruits was essentially maintained throughout the day. This decoupling of assimilation and stomatal conductance of fig fruits provides novel evidence that the coadaptation and inherent tradeoffs associated with the intricate relationship of fig fruiting cycles and life cycles of wasps extend into the fig and wasp physiologies by determining the internal temperatures of developing fig fruit and the maximum temperatures that the wasps developing in the fruit can endure.

Population Biology of Common 'Amakihi, *Hemignathus* sp., Inferred Using Allelic Genealogies[27]

LESLIE J. DOUGLAS[28]

A rather new emphasis in evolutionary genetics is the use of DNA sequence polymorphisms to reconstruct parameters of population growth. Mitochondrial DNA sequences from 66 individual birds were collected, corresponding to the same 250 base-pair region of the cytochrome B gene. Birds from Maui and Hawai'i, as well as O'ahu and Kaua'i, were included in this study to examine patterns of lineage diversification and dispersal. Phylogenetic analysis using distance, parsimony, and likelihood methods was performed, and average pairwise distances (APD) for each island population of lineages were calculated. The largest APD appeared among the lineages collected

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[27] Supported by a MacArthur Foundation grant to Leonard Freed, Rebecca Cann, and Sheila Conant.

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from Kaua'i, reflecting either a more ancient population or possible recent hybridization events between different species within the genus. The smallest APDs were between Maui and Hawai'i, indicating either historical or ongoing gene flow. O'ahu birds appear distinct in all analyses, grouping as a single cluster with APD values intermediate between the Maui-Hawai'i and Kaua'i lineages. Recent declines in Hawaiian bird population sizes due to habitat loss and introduced diseases have not yet dramatically reduced genetic variation at the mtDNA cytB locus. This result emphasizes the time lag necessary for detecting the loss of polymorphism in a species that has previously experienced rapidly expanding populations.

Identification and Physiological Characterization of Light Organ Symbionts of Mediterranean Sepiolid Squids[29]

PAT M. FIDOPIASTIS[30] AND SIGURD VON BOLETZKY[31]

Two genera of sepiolid squids (*Euprymna*, found primarily in warm, shallow coastal waters off Hawai'i and Australia, and *Sepiola*, the colder, deeper-water [>50 m] dwelling Mediterranean squids) are known to recruit luminous bacteria into light organ symbioses. The light organ symbiont of *Euprymna* spp. has been reported in recent years to be *Vibrio fischeri* (Beijerinck, 1889), but until now none of the symbionts of any *Sepiola* spp. has been identified. Preliminary microscopic and biochemical analyses of bacteria isolated from the light organs of *S. affinis* and *S. robusta* (Naef, 1912), whose light organ symbionts are designated "SA" and "SR", respectively, suggested that they were either *V. fischeri* or the closely related marine luminous bacterium *V. logei* (Bang, 1978). In this study, we identified the light organ symbionts of both *S. affinis* and *S. robusta* using DNA sequence comparisons, and performed experiments to determine how these strains compare physiologically with type strains of *V. fischeri* and *V. logei*. The 16S rDNA sequences of *V. fischeri* and *V. logei* are almost identical except in the region of bases 68–106, a variable region designated V1. *V. fischeri* strains yield a 120–121 bp fragment when a portion of the 16S rDNA containing the V1 region is amplified using the polymerase chain reaction. In contrast, *V. logei* type strains, as well as most SA and SR strains, yield a smaller 111–113 bp fragment. When we sequenced a ± 300 bp portion of the 16S rDNA from SA and SR strains, most of the sequences aligned with highest identity (>97%) to *V. logei*. These predominant SA and SR strains also exhibited physiological traits characteristic of *V. logei*: a reduced growth rate at 28° C relative to that at 20° C; and a reduced luminescence at higher temperatures, which was due to aldehyde substrate limitation. Molecular and physiology data taken together support our conclusion that the predominant light organ symbionts of at least two species of *Sepiola* are *V. logei*; it is interesting that some light organs also contained *V. fischeri* cells as 10–20% of their symbiont population. *Vibrio logei* is thus the first new addition in 20 yr to the list of luminous bacterial species involved in forming a light organ symbiosis, and these *Sepiola* species are the first bioluminescent animals to be shown to harbor mixed populations of two light organ symbionts.

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Bacteria-Induced Morphogenesis of the *Euprymna scolopes* Light Organ[32]

JAMIE S. FOSTER[33]

The symbiotic association between the Hawaiian sepiolid squid *Euprymna scolopes* Berry and the luminescent bacterium *Vibrio fischeri* has emerged as an experimentally tractable model system for the study of the influence of bacteria on animal development. In the adult host squid, the bacterial symbionts are housed extracellularly within a complex bilobed light-emitting organ in the center of the mantle cavity. The symbiosis is established anew each generation, and the juvenile light organ has a markedly different morphology from that of the adult. My research program focuses on determining the extent of bacterial involvement in these developmental changes. Previous research had shown that during the infection process, free-living *V. fischeri* are swept into pores on the surface of the juvenile light organ by a superficial, ciliated field that covers the lateral faces of the light organ. In response to *V. fischeri*, the organ undergoes extensive cell death of the ciliated epithelial field. Herein I report results of studies in which I have analyzed the underlying ultrastructure and biochemical features of this early cell death process. Transmission electron microscopy and confocal microscopy of the hatchling light organ revealed that the appendages of the ciliated field are composed of a single layer of epithelial cells overlying a sinus. Within 24 hr following exposure to the bacteria, the basement membrane of the epithelium had lost its integrity and the sinus had completely collapsed. The dying cells exhibited condensation and margination of the chromatin toward the periphery of the nucleus. In addition, we have used specific biochemical markers of cell death to characterize these bacterial-induced cell death events, including labeling of DNA fragments and assays of enzyme activities characteristic of cell death. These ultrastructural and biochemical analyses provide evidence that *V. fischeri* induces apoptotic cell death, not necrosis of the superficial epithelium of the light organ, during the initiation phase of symbiosis.

Tako Tastes Better than Calamari: Cephalopod Diet of the Hawaiian Monk Seal

GWEN GOODMAN-LOWE[34]

Hawaiian monk seal feces were collected between 1991 and 1994 from beaches of the Northwestern Hawaiian Islands (NWHI). The diet determined from fecal analysis comprised approximately 65% teleosts, 25% cephalopods, and 10% crustaceans. The cephalopod prey consisted of 22% octopus and 3% squid. Cephalopods were identified to species using upper and lower beaks obtained from known specimens. Five benthic and two pelagic species of octopus were identified, representing a mix of diurnally and nocturnally active species. In addition, 19 species of squid were found in the samples, representing a mix of coastal, pelagic, and mesopelagic species. These findings contrast with the previously held belief that Hawaiian monk seals are primarily inshore, nocturnal foragers, and indicate that cephalopods are an important component in the diet of this highly endangered species.

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Assemblage Structure and Species Richness of 'Ōhi'a (*Metrosideros polymorpha*) Canopy Arthropods as Sampled by Fogging: Preliminary Results and Future Directions[35]

DANIEL S. GRUNER[36]

The surge of interest in the last decade concerning estimation of global species richness results largely from insecticidal fogging studies of arboreal arthropods in the Tropics. I used quantitative fogging techniques to sample the canopy arthropod fauna of 'ōhi'a (*Metrosideros polymorpha* Gaudichaud-Beaupre) in Volcanoes National Park on the island of Hawai'i in October 1996. 'Ōhi'a is the dominant tree in many habitat types across all the major Hawaiian islands, thus offering opportunities for replicable comparisons of community structure across historical and ecological gradients. In this exploratory study, a total of 14 trees was selected to represent ranges of precipitation, volcanic substrate age (200–10,000 yr B.P.), and elevation, factors that vary independently and were expected to affect arthropod community composition. Species richness varied positively as a function of tree height and substrate age, but not of precipitation or elevation. Species richness was strongly positively related to the interaction of precipitation and substrate age. A greater percentage of adventive arthropods was found in association with introduced ants than in their absence. Comparisons with the late Wayne Gagné's *Metrosideros* fogging data from the International Biological Program in the 1970s show new arthropod invasions in the last 20 yr.

Anomalous Conduction in *Shaker* B K^+ Channels: Conduction in "Nonconducting" Mutants with Modified S4 Segments

MARK HENTELEFF, M. D. RAYNER, J. G. STARKUS, H. BAO, A. HAKEEM, AND M. ANDRES[37]

In N-terminus deleted *Shaker* B channels, the W434F pore domain mutation typically prevents permeation by potassium ions. We describe here S4 mutations that modify the action of W434F, such that K^+ currents remain although channel gating becomes voltage-insensitive. In a series of S4 mutants with multiple charge-neutralizations, we found voltage-sensitive gating with parameters similar to channels with unmodified S4 segments. In mutants from this series in which either charges 1 and 2 or charges 1, 4, and 7 were neutralized, addition of W434F resulted in maintained K^+ currents in which conductance is unaffected by changes in membrane potential between −80 and +80 mV. However, back mutation from glutamine to arginine at the first charge site restores both normal voltage-sensitive gating and the expected "nonconducting" behavior of the W434F mutation. We conclude that the W434 residue normally interacts both with the permeation pathway and with charges toward the N-terminus end of the S4 segment. The W434 residue may thus be an important component of the mechanism by which channels are "gated" into conducting or nonconducting states by S4 movement.

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Effects of UV Radiation and Water Flow on the Physiology of the Reef Coral *Porites compressa* and Tissue Concentration of UV-Absorbing Compounds[38]

ILSA B. KUFFNER[39]

Effects of UV radiation and water flow on several physiological parameters of *Porites compressa* Dana were explored in a manipulative $2 \times 2 \times 2$ factorial experiment. The purpose of the study was to explore the hypothesis that mycosporine-like amino acids (MAAs) are produced as sunscreens by the coral-algal symbiosis in response to UV radiation. Branches of *P. compressa* were cemented to tiles in growth position and subjected to treatments in a flow-through sea table flume for 6 weeks. Corals were exposed to two levels of water motion (15 cm/sec and 3 cm/sec) and two levels of UV radiation (ambient and none). Three-way analysis of variance was performed on the data set to reveal the effects of UV, water flow, duration of the treatment, and any interactions among these factors on the concentration of MAAs, calcification rate, and chlorophyll *a* concentration. The level of UV radiation was a significant determinate of MAA concentration; corals incubated without UV radiation gradually lost their MAAs. The concentration of chlorophyll *a* increased significantly in corals receiving high water motion, but the concentration of MAAs was not affected by flow regime. This evidence supports the putative photoprotective role of MAAs in the coral-algal symbiosis.

Broadband Recording of Free-Ranging Delphinid Social Acoustic Signals[40]

MARC O. LAMMERS[41] AND WHITLOW W. L. AU[42]

Over the past three decades a number of workers have analyzed and characterized the social and communication signals of several species of delphinids. Most of this research has employed band-limited recording equipment and has focused on the sonic components of the signals. As a result, most discussions of delphinid whistles and nonecholocation pulsed signals have been limited almost exclusively to below approximately 20 kHz. A technique has been developed that allows collection of high-fidelity recordings of signals up to 55 kHz in frequency. By digitizing signals directly into a laptop computer through a PCMCIA A/D converter, the sonic and ultrasonic components of the whistles and burst pulses of two populations of Hawaiian spinner and spotted dolphins (*Stenella longirostris* and *S. attenuata*, respectively) resident along the leeward coast of O'ahu, Hawai'i, are being studied. Preliminary results show that the ultrasonic range above 20 kHz is rich with whistle harmonics and components of burst-pulsed signals.

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Development and Utility of an Acoustic Tailbeat-Sensing Transmitter[43]

CHRISTOPHER G. LOWE, KIM HOLLAND, THOMAS WOLCOTT, AND JIM MCKIBBEN[44]

An acoustic tailbeat-sensing transmitter was developed to accurately determine activity rates, swimming speeds, and metabolic rates of free-swimming fishes and sharks. This device operates on a simple CMOS circuit and is triggered by the lateral movement of the animal's caudal fin. The transmitter can be quickly attached using dissolvable sutures or surgical staples and weighs 25 g in air and less than 2 g in seawater. Laboratory measurements of swimming speed and oxygen consumption as a function of tailbeat frequency can be used to predict activity rates and energy consumption of free-swimming instrumented fishes and sharks in the field. Preliminary tracks of hammerhead shark pups indicate that the shark's activity rates differed between day and night, with peaks in activity occurring during crepuscular periods. Shark pups exhibited an average diel swimming speed of 1.9 ± 0.34 (SD) km hr^{-1} and burned an estimated 29 ± 4 Kcal day^{-1}. Development of this minimally intrusive, activity-sensing transmitter provides a cost-efficient, accurate method for determining energy consumption of active fishes and sharks in the field.

Exploring the Microenvironment of the Symbiotic Light Organ of *Euprymna scolopes*[45]

SPENCER V. NYHOLM[46]

In any animal/bacterial mutualism the host must prevent uncontrolled symbiont growth while providing resources, such as nutrition, to foster a healthy bacterial population. The light organ symbiosis of the Hawaiian bobtail squid, *Euprymna scolopes* Berry, offers a unique opportunity to investigate questions concerning this balance. My research focuses on how the biochemical environment surrounding the symbionts is involved in host-mediated regulation of this mutualism. The squid houses the extracellular bioluminescent bacterium *Vibrio fischeri* in a bilobed light organ within complex crypts lined with a simple columnar epithelia. One way in which the squid controls symbiont number is through a light-cued diel venting of 90% of the bacterial population. We have been able to take advantage of this behavior to induce venting of the bacterial population and to collect the contents of the light organ for further analysis. My studies show that the crypt contents emerge as a "pastelike" exudate from the pores of the light organ. Electron microscopy revealed that this material consists of a mixture of animal and bacterial cells embedded in a thick matrix. Biochemical studies using gel-filtration chromatography, reverse-phase HPLC, and SDS gel electrophoresis showed that the matrix material, which represents the immediate microenvironment surrounding the symbionts, is

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composed of a complex mixture of proteins and peptides. I am currently examining this milieu of proteins to determine their origin (animal or bacterial) and whether a subset of these may be the source of nutrition provided to the bacterial symbionts by the host. I am also analyzing these proteins for other biochemical properties that may be involved in maintaining the balance of symbiont number in this dynamic microenvironment.

Morphology and Life Cycle of an Undescribed *Ophryotrocha* Species (Polychaeta: Dorvilleidae) and Its Possible Role as an Indicator of Organic Enrichment[47]

Brian L. Paavo[48]

A small (<2 mm) undescribed polychaete (Dorvilleidae) species has been regularly collected during an ongoing biomonitoring program off the Sand Island sewage outfall since 1987. A life history table has been constructed from observations of several generations reared at 26° C and fed combinations of *Enteromorpha intestinalis*, flake food, and powdered spinach. Several specimens of various ages were prepared for light and scanning electron microscopy. Consideration of reproductive events and certain parapodial, maxillary, and ciliary characteristics revealed that specimens represent a new species within the neotenic genus *Ophryotrocha*. A literature search and correspondence with polychaete researchers indicate that mention of this species has appeared in publication, but it has not been formally described. A formal description is being prepared. Trends in abundance variation in relation to distance from the sewage outfall were corroborated during the Māmala Bay Study Project. The two benthic studies have concluded that the *Ophryotrocha* sp. is a potential indicator species for organic enrichment. Several aspects of the life history of the *Ophryotrocha* sp. make it a prime candidate for experimental studies. These include a short generation time (ca. 30 days), predictable behaviors related to mating and parental care, an easily observed specific host-parasite relationship with a protozoan parasite, and recent construction of a gene library for genetic studies.

Male Mate Choice in the Monogamous Butterflyfish *Chaetodon multicinctus*

David A. Strang[49]

In the mating system of many animals, it is typically the female that most often exhibits mate choice. This is because females generally have a greater reproductive investment, both in the production of energetically expensive eggs and also in the care of the offspring. Males, on the other hand, produce energetically less-expensive sperm and so tend to have a lower reproductive invest-

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ment. This leads to different tactics between males and females for increasing their reproductive success. Females attempt to increase their reproductive success by investing in egg production, offspring care, and in the choice of a quality mate, whereas males attempt to increase their reproductive success by competing with other males for matings with multiple females. In a monogamous species, however, the male and female of a pair share the same reproductive success and are likely to have similar levels of reproductive investment. Therefore, males and females of monogamous species are expected to invest more equally in the choice of a quality mate. *Chaetodon multicinctus* is a monogamous butterflyfish that defends territories of coral upon which it feeds. Monogamy in this species seems to be related to the even distribution of females on the reef and the low opportunities for males to find and keep multiple mates. I tested the effect of fish size on mate choice in *Chaetodon multicinctus* in an effort to see if both males and females are selective about their mates. Males may be less selective if they still have a lower reproductive investment or if they have the opportunity for matings outside the pair.

Chronology of the Dawn Chorus in Hawaiian Forest Birds

ERIC A. VANDERWERF[50]

The dawn chorus is a peak in singing activity that occurs just before sunrise and is common in many birds. Previous studies have focused on the proximate cues that trigger song and on the adaptive functions of the dawn chorus, but there has been little work on how singing might vary among species or change over time. I determined the time of first song in the dawn chorus for eight species of birds on the island of Hawai'i on 115 mornings over a 17-month period. Correlation of song time and sunrise time was very high for all species ($r = 0.94$–0.99), demonstrating that birds relied primarily on light as a cue to begin singing. Mediating effects of species, weather, nesting stage, and their interactions were investigated with an analysis of variance using song time relative to sunrise to remove the effect of sunrise. Species began singing at different times, but in a consistent order, and the order varied seasonally according to the breeding cycle of each species. Birds sang earlier relative to sunrise during their nesting season, and the magnitude of the difference was greater for sexually dimorphic species, suggesting that song time is sexually selected. Singing started later on cloudy and rainy mornings, presumably because light intensity was lower than on clear days. Rain affected song time less in the nesting season, suggesting that birds are under greater pressure to sing early while nesting regardless of environmental conditions. Effect of temperature was investigated separately because of nonindependence with weather. Birds sang earlier on colder mornings, supporting the hypothesis that singing activity is greater when attenuation and degradation of sound by solar radiation are minimal. For one species, the 'elepaio, song playbacks induced singing earlier than usual in the nesting season, but not in the nonnesting season, indicating that there is also a social component to the seasonal timing of the dawn chorus.

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Lipid Composition of Deep-Sea Sharks from the Chatham Rise, New Zealand

BRAD WETHERBEE[51]

Deep-sea sharks approach neutral buoyancy by means of a large, oily liver that contains large amounts of low-density lipids: primarily squalene and diacyl glycerol ether (DAGE). As an animal increases in size and matures sexually, many biochemical changes take place within the animal. It was hypothesized that maintenance of neutral buoyancy in deep-sea sharks involves fine-scale changes in the chemical composition of liver oil as individuals grow and develop. To test this hypothesis the lipid composition of liver oil of individuals of different sizes and sex for several species of deep-sea sharks collected from the Chatham Rise, New Zealand, was compared. The composition of liver oil varied between and among species. Several species contained large amounts of squalene and DAGE, whereas only traces of these lipids were present in other species. There was an inverse relationship between the amount of squalene and DAGE in liver oil, and squalene content tended to decrease as sharks increased in size. The species with liver lipid characteristics most desirable for commercial exploitation were not abundant on the Chatham Rise. Maintenance of neutral buoyancy in deep-sea sharks is a dynamic process that involves changes in the composition of low-density liver lipids as the sharks increase in size.

Tissue- and Stage-Specific Expression of the White Eye Gene in the Oriental Fruit Fly, *Bactrocera dorsalis*

JINGWEI XIAO, SUSAN D. MCCOMBS, JINSHENG YANG, AND STEPHEN H. SAUL[52]

Total RNA and mRNA from different tissues and stages of the wild-type and white eye (*we*) mutant strains were subjected to Northern analysis. The RNA probe for detecting expression of the *we* gene was prepared from a 700-bp DNA fragment that extends from exon 4a to exon 6 of the *we* gene in *Bactrocera dorsalis* (Hendel). The transcript of the wild-type *we* gene was present in all stages, with highest expression in the mature third instar. The *we* transcript was detected in the eye and Malpighian tubules of larvae and adults as well as in adult testes. The *we* mRNA was not found in the third instar of the mutant strain; however, a transcript was detected in all adult tissues. The transcript in the mutant adult was shorter (1.9 kb) than that detected in the wild-type (3.5 kb). The transcript of the wild-type *we* gene of *Bactrocera cucurbitae* (Coquillett) is also 3.5 kb; however, the same gene in *Ceratitis capitata* (Wiedemann) and *Drosophila melanogaster* (Meigen) is smaller, at 2.6 kb. Our sequence analysis did not indicate deletions or altered mRNA splicing pattern within the major coding region, from exon 2 through exon 6. Therefore, our future work will focus on sequence comparison of exon 1 and upstream regulatory regions to determine the basis of the *we* mutation in *B. dorsalis*.

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A Novel Approach to the Study of Structure-Function Relationships in the Sodium Ion Channel

ANGEL ANNE YANAGIHARA, MARK HENTELEFF, AND MARTIN RAYNER[53]

Elucidation of the structure-function relationships in the four-domain, voltage-gated sodium ion channel is an area of rich convergence of the fields of classical biophysics, biochemistry, and molecular biology. This study aimed to test the ability of the N- and C-terminal flanking regions of the transmembrane voltage-gated potassium ion channel to direct the tetrameric assembly of domain I (DI) of the voltage-gated sodium ion channel. Specifically, the DI of the voltage-gated sodium ion channel of rat skeletal muscle (SkM) was excised and inserted between the N- and C-terminal coding regions of an inactivation removed type-A voltage-gated potassium ion channel, *Shaker* B 29-4 IR. The biophysical and biochemical properties of this novel chimeric construct will be investigated following expression in *Xenopus laevis* oocytes. Potential outcomes of this proposed research strategy include confirmation of the putative tetrameric assembly role ascribed to the N-terminus of ShB, establishment of a valuable experimental model for further characterization of DI-specific structural features, and facilitation of the study of the other domains of SkM.

Selective Down-Regulation of T-Cell Receptor Vβ6 Gene Expression in Tuberculin Skin Test–Reactive Individuals[54]

CHAOQUAN YIN, VIVEK R. NERURKAR, LUCILLE KIMURA, WAN-MOHAIZA DASHWOOD, AND RICHARD YANAGIHARA[55]

Recent studies, indicating that progression to AIDS occurs more rapidly among HIV-1 infected tuberculin skin test–reactive individuals and reciprocal enhancement of HIV-1 replication in monocytoid and lymphocytoid cells coinfected with *Mycobacterium tuberculosis* (MTB), are particularly relevant to Hawai'i, which ranks first nationally in the incidence of TB. To determine if specific T-cell receptor (TCR) Vβ genes are differentially expressed in MTB infection, we employed semiquantitative RT-PCR to analyze RNA extracted from unstimulated and PHA-stimulated PBMC and flow cytometry to analyze PHA-stimulated PBMC obtained from 18 healthy adults (age range, 22–55 yr), who were either exposed or naive to BCG vaccination and either reactive or nonreactive to PPD skin test. Size-fractionated amplified products were visualized by ethidium bromide–staining, and the Vβ band intensities were compared with the internal Ca control band and quantitated using the FOTO/Analyst Image Analysis System and Collage version 4.0, and selective TCR Vb repertoires were flow cytometrically analyzed by using monoclonal antibodies to TCR b chain variable regions. Our data indicate that TCR Vβ6 repertoires is significantly down-regulated in unstimulated and PHA-stimulated PBMC from BCG-exposed, PPD-reactive individuals, when compared with that of BCG-naive and PPD-nonreactive healthy individuals ($n = 6$).

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